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(71) Applicant: HEMAGEN/PFC [US/US]; 11810 Borman Drive, St. Louis, MO 63146 (US).			
(72) Inventors: CACHERIS, William, P.; 16726 Edisto Court, Florissant, MO 63034 (US). KAUFMAN, Robert, J.; 8129 Stanford Avenue, University City, MO 63130 (US). RICHARD, Thomas, J.; 2381 Fairroyal Drive, Des Peres, MO 63131 (US). GRABIAK, Raymond, C.; 2016 Rule Avenue, Maryland Heights, MO 63403 (US).			
(74) Agents: JOSEPHIC, David, J. et al.; Wood, Herron & Evans, 2700 Carew Tower, Cincinnati, OH 45202 (US).			
(54) Title: EMULSIONS OF PARAMAGNETIC CONTRAST AGENTS FOR MAGNETIC RESONANCE IMAGING (MRI), CONTAINING AN ORGANIC CHELATOR HAVING AN UNSATURATED ALIPHATIC GROUP			
(57) Abstract			
Emulsions of paramagnetic contrast agents and processes of making and using them are disclosed. The emulsions contain water, a dispersed oil phase and a complex of a paramagnetic metal ion and an organic chelator having a C ₁₀ -C ₃₀ unsaturated aliphatic group. The emulsions are very stable and therapeutically acceptable for intravenous administration to enhance MRI.			

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EMULSION OF PARAMAGNETIC CONTRAST AGENTS FOR MAGNETIC RESONANCE IMAGING (MRI), CONTAINING AN ORGANIC CHELATOR HAVING AN UNSATURATED ALIPHATIC GROUP

TECHNICAL FIELD OF THE INVENTION

This invention relates to emulsions of paramagnetic contrast agents, and processes of making and using them. More particularly, this invention relates to novel emulsions that contain water, a dispersed oil phase and a complex of a paramagnetic metal ion and an organic chelator having a C₁₀-C₃₀ unsaturated aliphatic group. The emulsions are very stable and are therapeutically acceptable for intravenous administration to enhance MRI imaging.

BACKGROUND OF THE INVENTION

Magnetic resonance imaging (MRI) has been developed in recent years and, for improved imaging, paramagnetic contrast agents have been given to patients prior to imaging. A number of patents disclose paramagnetic MRI contrast agents including, for example, U.S. Patents 4,647,447; 4,859,451; 4,957,939; 4,963,344; 5,021,216; 5,064,636 and 5,120,527; and PCT application WO 92/21017. These patents are considered to be illustrative of prior references in the field and are not intended to be the most pertinent references.

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Paramagnetic agents of the type disclosed in the above patents have been administered to the patient in the form of aqueous solutions. In addition, paramagnetic oil emulsions have been provided for MRI imaging in the 5 gastro-intestinal tract as disclosed in U.S. Patents 5,064,636 and 5,120,527. There has been a continuing effort to develop complexes of paramagnetic metal ions as 10 MRI contrast agents that function effectively as organ imaging agents as well as blood pool agents, or for other uses, such as agents for imaging the bone marrow, spleen, liver or lymph nodes. Liposomes have also been studied as 15 MRI contrast agents, and, more recently, as disclosed in PCT application WO 92/2107, lipo soluble contrast agents may be administered in the form of lipid emulsions. The contrast agents of the PCT application are useful in the imaging of the liver, blood pool and reticuloendothelial system (RES).

Notwithstanding the prior efforts in the field, there is a continuing need for improved MRI contrast 20 agents. In particular, MRI contrast agents are needed which function effectively as organ imaging agents as well as blood pool agents, and for general imaging of the reticuloendothelial system. Stable and versatile MRI contrast agents are needed, especially for intravenous 25 use.

SUMMARY OF THE INVENTION

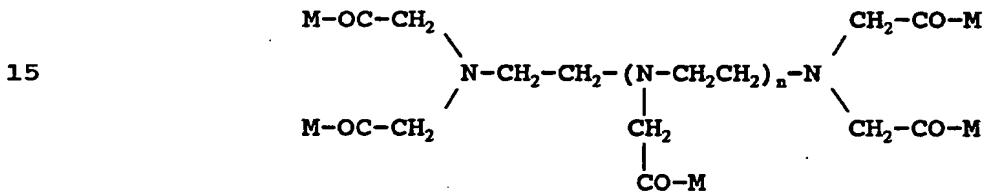
This invention is directed to a physiologically acceptable emulsion for enhancement of MRI imaging

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comprising water, a dispersed oil phase and a paramagnetic metal chelate complex having a C₁₀-C₃₀ unsaturated aliphatic group. It has been discovered that very fine and stable emulsions may be made using such chelate complexes. These
 5 emulsions remain stable after heat sterilization. Moreover, these emulsions may be used intravenously and provide excellent MRI images.

More particularly, the physiologically acceptable emulsion for enhancement of MRI imaging
 10 comprises water, a dispersed oil phase and a complex of a paramagnetic metal ion and an organic chelator having the formula



20 wherein from 2 to 5 M groups are hydroxyl, n=0 to 2, and any remaining M group is NR₁R₂, each R₁ is a C₁₀-C₃₀ unsaturated aliphatic group and R₂ is hydrogen, hydroxyl or a C₁-C₂ alkyl.

25 The metal ion is a lanthanide element of atomic numbers 58-70 or a transition metal of atomic numbers 21-29, 42 or 44, most prefereably selected from a group consisting of Gd(III), Mn(II), iron and dysprosium. The organic chelator is preferably an acid selected from the group consisting of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid. Mono- or bis(amides)

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having an unsaturated C₁₄-C₂₂ group are physiologically preferred. It has been established that the unsaturated group may have one, two, three or more double bond(s) at a number of different locations in the group, and very
5 fine, stable emulsions are still achieved.

The MRI emulsions for intravenous administration have an average particle size less than about 1 micron, preferably on the order of about 0.2 to about 0.4 micron. In other embodiments, the emulsions
10 comprise water, a dispersed oil phase selected from the group consisting of an oil and a fluorochemical, and mixtures thereof, a surfactant, and a dispersed complex of a paramagnetic metal ion and an organic chelator. The emulsified particles of an oil and/or a fluorochemical
15 ("PFC") in water are hereinafter sometimes referred to as the "dispersed oil phase". The paramagnetic agent may be effectively suspended or dispersed in the stabilized emulsion for delivery to an animal or human subject.

In contrast to prior MRI agents and
20 compositions, the MRI emulsions of this invention are very stable and exhibit excellent storage stability at room temperature or other ambient conditions. Furthermore, the inventive emulsions produce excellent MRI images of organs, blood pool and the RES.

25 This invention also includes methods of making emulsions containing paramagnetic agents. Other objectives of this invention and advantages will become apparent from the following detailed description.

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DETAILED DESCRIPTION OF THE INVENTION

The MRI emulsions of this invention comprise an oil and/or a fluorochemical (PFC) emulsified in water and contain a paramagnetic metal chelate complex. In certain cases, the chelate complex may act as a surfactant and, thus, additional cosurfactant may not be needed. In most cases, a surfactant is added. In general, the oil and/or PFC may be contained in amounts from about 0.5 to 50% by weight. More specifically, for instance, in medical applications for intravenous (IV) MRI contrast agent delivery, the preferred amounts of PFC and/or oil with surfactant are minimum amounts to effectively disperse the agent in a stable emulsion. For oral, rectal, or other delivery, far greater amounts may be desirable. For IV use, about 25 w/v% is a practical limit for the oil, or about 55 v/v% for the PFC, because of viscosity limitations for an intravenous product. Preferred ranges are about 5 to 20 w/v% for the oil or about 5 to 50 v/v% for the PFC. Emulsions exhibit high viscosity (or a gel-like consistency) at higher oil or PFC levels. The surfactant may be contained in amounts from about 0.5 to about 10% by weight, usually about 1-5% by weight of the emulsion. Generally, the MRI agent may be dispersed in varying amounts up to about 30% by weight, depending upon dose, efficacy and safety requirements. Thus, an IV emulsion may preferably contain a lesser amount of MRI agent up to about 10% by weight. For instance, in oral or rectal administration, an MRI imaging agent such as a

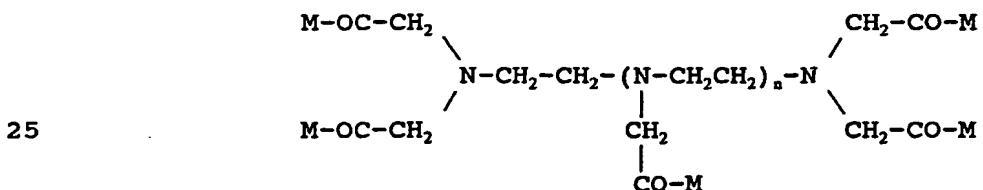
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gadolinium salt of a bis(oleylamide) of diethylenetriaminepentaacetic acid may be used as high as about 50% or more. If desired, the emulsions may be diluted with isotonic saline, or other agents, to produce lower concentrations. These components are identified with greater particularity as follows.

A. Paramagnetic Metal Chelate Complex

In a broad form, this invention is directed to
a physiologically acceptable emulsion for enhancement of
MRI imaging comprising water, a dispersed oil phase and a
paramagnetic metal chelate complex having a C₁₀-C₃₀
unsaturated aliphatic group. It has been discovered that
very fine and stable emulsions may be made using such
chelate complexes. Moreover, these emulsions may be used
intravenously and provide excellent MRI images.

More particularly, the physiologically acceptable emulsion for enhancement of MRI imaging comprises water, a dispersed oil phase and a complex of a paramagnetic metal ion and an organic chelator having the formula



wherein from 2 to 5 M groups are hydroxyl, n=0 to 2, and any remaining M group is NR₁R₂, each R₁ is a C₁₀-C₂₀

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unsaturated aliphatic group and R₂ is hydrogen, hydroxyl or a C₁-C₂ alkyl.

The metal ion is a lanthanide element of atomic numbers 58-70 or a transition metal of atomic numbers 21-5 29, 42 or 44, most preferably selected from a group consisting Gd(III), Mn(II), iron and dysprosium. The organic chelator is preferably an acid selected from the group consisting of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid. Monoamides or 10 bisamides of an organic acid selected from a group consisting of diethylenetriaminepentaacetic acid and ethylenediaminetetraacetic acid are used wherein each R₁ is a C₁₄-C₂₂ group selected from the group of oleyl, farnesyl, geranyl, erucyl, elaidyl, linoleyl, ricinoleyl, 15 petroselinyl, linolenyl, vaccenyl, linderyl, palmitoleyl, palmitelaidyl, myristoleyl, and myristelaidyl. The N-methyl, N-ethyl, and N-OH (where R₂ = methyl, ethyl, or OH for each R₁) derivatives of these amides can also be included as examples.

20 Specific examples of chelate complexes include gadolinium diethylenetriaminepentaacetic acid bis(oleylamide), gadolinium diethylenetriaminepentaacetic acid mono(oleylamide), gadolinium diethylenetriaminepentaacetic acid bis(farnesylamide), 25 gadolinium diethylenetriaminepentaacetic acid bis(geranyl amide), gadolinium diethylenetriaminepentaacetic acid bis(erucylamide), gadolinium diethylenetriaminepentaacetic acid

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bis(elaidyl amide), gadolinium diethylenetriaminepentaacetic acid bis(linoleylamide), gadolinium diethylenetriaminepentaacetic acid bis(ricinoleylamide), gadolinium 5 diethylenetriaminepentaacetic acid bis(petroselinyl), gadolinium diethylenetriaminepentaacetic acid bis(N-ethyl-N-oleylamide), gadolinium diethylenetriaminepentaacetic acid bis(N-methyl-N-oleylamide), gadolinium diethylenetriaminepentaacetic acid bis(linolenylamide), 10 gadolinium diethylenetriaminepentaacetic acid bis(vaccenylamide), gadolinium diethylenetriaminepentaacetic acid bis(linderylamide), gadolinium diethylenetriaminepentaacetic acid bis(palmitoleylamide), gadolinium 15 diethylenetriaminepentaacetic acid bis(palmitelaidylamide), gadolinium diethylenetriaminepentaacetic acid bis(myristoleylamide), and gadolinium diethylenetriaminepentaacetic acid bis(myristelaidylamide). The gadolinium complexes of the 20 N-methyl, N-ethyl, and N-OH (where R₂= methyl, ethyl, or OH for each R₁) derivatives of these amides can also be included as examples.

B. Oil

The term "oil" is used herein in a general sense 25 to identify a large class of physiologically acceptable substances whether of mineral, vegetable, animal, essential or synthetic origin. Thus, the term "oil" is used herein as applied to a wide range of substances that

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are quite different in chemical nature. In the classification of oils by type or function, for example mineral oil is derived from petroleum and includes aliphatic or wax-based hydrocarbons, aromatic hydrocarbons or mixed aliphatic and aromatic based hydrocarbons. Also included in the mineral classification are petroleum-derived oils such as refined paraffin oil, and the like. In the vegetable classification, oils are chiefly derived from seeds or nuts and include drying oils such as linseed and tung oil; semidrying such as safflower and soy bean oils; nondrying such as castor, cottonseed and coconut oils and edible soap stocks such as palm and coconut oils. In the animal classification, oils usually occur as fats in tallow, lard and stearic acid sources. The liquid animal types include fish oils, oleic acid, sperm oil, etc. and they usually have a high fatty acid content. Included are some vegetable oils, such as olive, cottonseed, corn and peanut, as well as some special fish oils such as cod-liver, haliver, shark liver, and so forth which are used largely as medicines for their high vitamin content. A liquid fatty oil such as a mono-, di-, or triglyceride, or a mixture thereof, is the preferred oil. Medium chain triglycerides also serve as useful oils according to this invention.

25 C. Fluorochemical

In this description, "fluorochemical" or "PFC" is used to describe either a highly fluorinated organic compound of perfluorocarbon or fluorinated chemical.

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Further, these terms are used interchangeably. The term "perfluorocarbon" includes a "cyclic" or "acyclic" compound of carbon. Substituted derivatives thereof are also included where fluorocarbons have other elements within their structures such as oxygen, hydrogen, nitrogen, chlorine, and bromine, etc. It should also be noted that the term "perfluorocarbon" is meant to include partially or substantially fluorinated compounds. This is permissible providing that the lack of complete replacement of all hydrogens does not affect the essential non-toxic characteristics of the preferred medical fluorocarbons of this invention. Among the perfluorocarbon compounds which may be employed are perfluorotributylamine (FC47), perfluorodecalin (PP5), perfluoromethyldecalin (PP9), perfluoroctylbromide, perfluorotetrahydrofuran (FC80), perfluoroether (PID) $[(CF_3)_2CFOCF_2(CF_2)_2CF_2OCF(CF_3)_2]$, perfluoroether (PIID) $[(CF_3)_2CFOCF_2(CF_2)_6CF_2OCF(CF_3)_2]$,

20 perfluoropolymer (E3) $[CF_3CHF(OCF_2CF)_2OCF_2CF_2CF_3]$,

perfluoropolymer (E4) $[CF_3CHF(OCF_2CF)_3OCF_2CF_2CF_3]$

25 perfluoroetherpolymer (Fomblin Y/01), perfluorododecane, perfluorobicyclo[4.3.0.]nonane, perfluorotritrimethylbicyclohexane, perfluorotripropylamine, perfluoroisopropyl cyclohexane, perfluoroendotetrahydronodicyclopentadiene,

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perfluoroadamantane, perfluoroexo-tetrahydrodicyclopentadiene,
perfluorobicyclo[5.3.0.]decane, perfluorotetramethylcyclohexane, perfluoro-1-methyl-4--
5 isopropylcyclohexane, perfluoro-n-butylcyclohexane,
perfluorodimethylbicyclo[3.3.1.]nonane, perfluoro-1-methyl
adamantane, perfluoro-1-methyl-4-t butylcyclohexane,
perfluorodecahydroacenaphthene,
perfluorotrimethylbicyclo[3.3.1.]nonane, perfluoro-1-
10 methyl adamantane, perfluoro-1-methyl-4-t
butylcyclohexane, perfluorodecahydroacenaphthene,
perfluorotrimethylbicyclo[3.3.1.]nonane, perfluoro-n-
undecane, perfluorotetradecahydrophenanthrene, perfluoro-
1, 3, 5, 7 - tetramethyl adamantane,
15 perfluorododecahydrofluorene, perfluoro-1-3-
dimethyladamantane, perfluoro-n-octylcyclohexane,
perfluoro-7-methyl bicyclo[4.3.0.] nonane, perfluoro-p-
diisopropylcyclohexane, perfluoro-m-
diisopropylcyclohexane, perfluoro-4-
20 methyloctahydroquinolidizine, perfluoro-N-methyl-
decahydroquinoline, F-methyl-1-oxadecalin,
perfluorooctahydroquinolidizine, perfluoro 5,6-dihydro-5-
decene, perfluoro-4,5-dihydro-4-octene,
perfluorodichlorooctane and perfluorobischlorobutyl ether.
25 Chlorinated perfluorocarbons, such as chloroadamantane and
chloromethyladamantane as described in U.S. Patent No.
4,686,024 may be used. Such compounds are described, for
example in U.S. Patent Nos. 3,962,439; 3,493,581,

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4,110,474, 4,186,253; 4,187,252; 4,252,824; 4,423,077;
4,443,480; 4,534,978 and 4,542,147, European Patent
Application Nos. 80710 and 158,996, British Patent
specification 1,549,018 and German Offen. 2,650,586. Of
5 course, it should be understood that mixtures of any of
these highly fluorinated organic compounds may also be
used in the emulsions and processes of this invention.

D. Surfactant

10 Surfactants are usually needed to form stable
emulsions indicated above where the MRI agent has
insufficient surfactant activity. Any suitable surfactant
may be employed alone or in combination with other
surfactants. For example, egg yolk phospholipids or
Pluronics emulsifying agents may be used. Pluronics
agents are block polymer polyols sold by Wyandotte, e.g.,
15 Pluronics F68, having a molecular weight of about 8,000,
may be employed. Ethoxylates of cholesterol, diacyl
glycerol and dialkyl ether glycerol are useful
surfactants. Also, using backbones of cholesterol, diacyl
20 glycerol or dialkyl ether glycerol, block copolymers are
made by adding ethylene oxide, propylene oxide and
ethylene oxide, in that order, in varying amounts to
produce surfactants. In some applications for
nonintravenous use, anionic or cationic surfactants may be
25 used. The emulsions of this invention may contain
alkylphosphoryl choline or alkylglycerophosphoryl choline
surfactants described in Kaufman and Richard, U.S. Ser.
No. 791,420, filed November 13, 1991. Specific examples

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of these surfactants are 1,2-dioctylglycero-3-phosphoryl choline, 1,2-ditetradecylglycero-3-phosphoryl choline, 1,2-dihexadecylglycero-3-phosphoryl choline, 1,2-di-
5 octadecylglycero-3-phosphoryl choline, 1-hexadecyl-2-tetradecylglycero-3-phosphoryl choline, 1-octadecyl-2-tetradecylglycero-3-phosphoryl choline, 1-tetradecyl-2-octadecylglycero-3-phosphoryl choline, 1-hexadecyl-2-octadecylglycero-3-phosphoryl choline, 1-2-
10 dioctadecylglycero-3-phosphoryl choline, 1-octadecyl-2-hexadecylglycero-3-phosphoryl choline, 1-tetradecyl-2-hexadecylglycero-3-phosphoryl choline, 2,2-ditetradecyl-1-phosphoryl choline ethane and 1-hexadecyl-tetradecylglycero-3-phosphoryl choline. The 1,3-dialkyl
15 Kaufman and Richard, U.S. Ser. No. 228,224, filed April 15, 1994 may also be used and the disclosure thereof is incorporated herein by references. Mixtures of these novel surfactants with other known surfactants may also be employed. Anionic surfactants include alkyl or aryl
20 sulfates, sulfonates, carboxylates or phosphates. Cationic surfactants include such as mono-, di-, tri- and tetraalkyl or aryl ammonium salts. Non-ionic surfactants include alkyl or aryl compounds, whose hydrophilic part consists of polyoxyethylene chains, sugar molecules,
25 polyalcohol derivatives or other hydrophilic groups. Zwitter-ionic surfactants may have a combination of the above anionic or cationic groups, and whose hydrophobic

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part consists of any other polymer, such as polyisobutylene or polypropylene oxides.

E. Emulsion Characteristics

The emulsions of this invention are made by dispersing the above ingredients in water and homogenizing them. The oil and/or PFC are dispersed in the water and enhance the dispersion of the paramagnetic metal chelate complex. The surfactant enhances the dispersion by stabilization of the liquid phases. While dispersions may be generally referred to herein as emulsions, it should be understood that they may be considered solutions, micellar solutions, microemulsions, vesicular suspensions, or mixtures of all of these physical states. The PFC may be dispersed in the oil and the oil-PFC phase emulsified in the water. However, other possible interfaces and phases are within the scope of the invention. Accordingly, the term "emulsion" as used herein covers all these states and the surfactant is employed to enhance stable mixtures of these physical states of the fluorochemical, oil, paramagnetic metal chelate complex and water phases. For example, a fluorochemical and oil may be emulsified in water, as described in the Clark and Shaw European Pat. Appln. 87300454.3 and this application is incorporated herein by reference to describe suitable PFC/oil emulsions as MRI delivery agents.

The MRI emulsions of this invention are very fine, stable emulsions. The criterion for a "fine" emulsion is no visible solid matter microscopically (300-

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400X) and less than 10 volume % of particles above about 0.8 μm ("CV"). The "poor" emulsions of comparative prior art, for example, have a large amount of huge ($>5 \mu\text{m}$) solids visible under the microscope, and greater than 10 volume % of particles above about 0.8 μm ("CV"). Reference is made to Figure 1 of the drawings which documents photographically the microscopic appearance of fine and poor emulsions at 300-400 X. In Figure 1, the fine emulsion contains 2% lecithin, 10% safflower oil and 5% GdDTPA-BOA of Example 8, Table 5, of this invention. The poor emulsion contains GdDTPA-BSA of Table 8 for comparison. Thus, these two complexes make markedly different quality emulsions although the complexes differ only in the unsaturation of the C₁₈ chain.

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BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts photographs of fine and poor emulsions at 300-400X.

Figures 1A and B are pre-contrast and post-
5 contrast photographic MRI images of brain and liver.

Figure 2 depicts pre-contrast and post-contrast photographic MRI images of a liver.

The following non-limiting examples illustrate the various embodiments of this invention.

10 General Procedure for DTPA Bis(amides)

Under a static nitrogen atmosphere or a CaSO_4 drying tube, a mechanically stirred mixture of diethylenetriaminepentaacetic (DTPA) dianhydride (1 mole) and anhydrous pyridine (2-24.7 mole; preferably 3.3 mole)
15 in chloroform (0-3 L/mole of DTPA dianhydride; preferably 1 L/mole) was treated dropwise with a solution of the appropriate amine (2 mole) in chloroform (0-2.5 L/mole of amine; preferably 0.25 L/mole). In some cases, a mild exotherm was apparent. The resulting mixture was then
20 heated at reflux (65°C w/o chloroform solvent) for 17-22 hours.

Workup Procedure A: After cooling to ambient temperature, the resulting reaction mixture was diluted with acetone (4-5 L/L of CHCl_3) and cooled to 0°C . The resulting solid was filtered and washed with acetone. In some cases, the resulting solid was purified further by recrystallization from appropriate solvent to give the corresponding DTPA Bis(amide) (See Table 1 for more

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details). These compounds were characterized by elemental analyses (see Table 2), infrared spectroscopy, proton and carbon nuclear magnetic resonance spectroscopy. High performance liquid chromatography was also used to assess 5 purity on some derivatives.

Workup Procedure B: If no precipitate was obtained with acetone dilution on a small aliquot, the resulting reaction mixture was washed with 5% HCl to remove the pyridine and then with saturated brine solution. After 10 drying over MgSO₄, concentration in vacuo yielded the crude DTPA Bis(amide) as a beige, glassy material (See Table 1 for more details). In some cases, column chromatography on silica gel with chloroform and methanol mixtures was used to remove trace impurities. These 15 compounds were characterized further by elemental analyses (see Table 2), infrared spectroscopy, proton and carbon nuclear magnetic resonance spectroscopy. High performance liquid chromatography was also used to assess purity.

The above procedures were used to make various 20 DTPA Bis(amide) ligands. The following Table 1 provides a variety of N R₁ R₂ groups under the above general formula in accordance with Workup A or B.

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Table 1. DTPA Bisamide Ligands

R ₁	R ₂	Workup Procedure	Recryst Solvent	Yield	mp (°C) ^a
Stearyl (C ₁₈ H ₃₇)	C ₂ H ₅ O	Chromatography	n/a	79%	168 (dec)
Oleyl (C ₁₈ H ₃₅)	C ₃ H ₇	Chromatography	n/a	96%	(glass)
Oleyl (C ₁₈ H ₃₅)	C ₄ H ₉	Chromatography	n/a	95%	(glass)
Oleyl (C ₁₈ H ₃₅)	H	A	EtOH	45%	150-175 ^b
Oleyl (C ₁₈ H ₃₅)	H	A	EtOH	75%	150-180 ^b
Oleyl (C ₁₈ H ₃₅)	H	A	None	91%	178-184 ^b
Stearyl (C ₁₈ H ₃₇)	CH ₃	A	EtOH ^c	81%	173-176
Stearyl (C ₁₈ H ₃₇)	CH ₃	A	EtOH ^c	89%	179-181
4-n-Hexadecylphenyl	H	A	DMF	97% ^d	190-194
Oleyl (C ₁₈ H ₃₅)	Oleyl (C ₁₈ H ₃₅)	B	Acetone	82%	115-150 ^b
n-Dodecyl (C ₁₂ H ₂₅)	H	A	MeOH ^c	96% ^d	180-183 ^e
Geranyl (C ₁₀ H ₁₇)	H	A	EtOH	91%	102-106
Farnesyl (C ₁₅ H ₂₅)	H	A	EtOH	75%	140-142
Stearyl (C ₁₈ H ₃₇)	H	A	AcOH/Et ₂ O	84%	155-158
Oleyl (C ₁₈ H ₃₅)	Hexyl (C ₆ H ₁₃)	B		86%	
Ercyl (C ₂₂ H ₄₃)	H	A	EtOH ^c	89%	168-172 ^c
Ricinoleyl (C ₁₈ H ₃₅ O)	H	A	EtOH ^c	91%	75-80
Petroselinyl (C ₁₈ H ₃₅)	H	A	EtOH ^c	96%	173-176 ^c
Elaidyl (C ₁₈ H ₃₅)	H	A	EtOH ^c	93%	173-178 ^c
Linoleyl (C ₁₈ H ₃₃)	H	A	EtOH ^c	91%	138-165
Oleyl (C ₁₈ H ₃₅)	OH			99%	

a. Melting points were not sharp and generally softened and sweated at a lower temperature. Melted with decomposition.
 b. Oleylamine and N,N-dioleylamine are invariably contaminated with the corresponding saturated amines (i.e. stearyl) and/or isomerized olefinic amines. These impurities presumably broaden the melting point.

c. In this case, the solid was simply slurried in hot solvent, rather than recrystallized to remove trace impurities, such as pyridine.
 d. Yield prior to recrystallization of an analytical sample.
 e. Analytical sample recrystallized from reagent alcohol.

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Elemental analysis of the DTPA bis(amide) ligands were also performed and reported in the following Table 2.

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Table 2. Elemental Analyses of DTPA Bisamide Ligands

R ₁	R ₂	Molecular Formula	Weight Per Cent Found (Calcd)			Water
			Carbon	Hydrogen	Nitrogen	
Oleyl	H	C ₅₀ H ₉₃ N ₅ O ₈ -0.2 H ₂ O	66.83 (67.03)	10.34 (10.51)	8.11 (7.82)	0.41 (0.41)
Stearyl	Methyl	C ₅₂ H ₁₀₁ N ₅ O ₈ -0.5H ₂ O ^a	67.00 (66.92)	11.14 (11.01)	7.47 (7.50)	0.89 (0.96)
Stearyl	Methyl	C ₅₂ H ₁₀₁ N ₅ O ₈ -1.0H ₂ O ^a	66.02 (66.27)	11.08 (11.02)	7.30 (7.43)	1.85 (1.9)
Stearyl	Stearyl	C ₄₆ H ₈₉ N ₅ O ₈ -1.0HCl-0.5H ₂ O ^b	62.00 (62.38)	10.13 (10.36)	7.66 (7.91)	0.52 (1.02)
4-n-Hexadecyl-phenyl	H	C ₅₈ H ₉₇ N ₅ O ₈ -1.0H ₂ O	68.94 (68.94)	9.52 (9.88)	6.86 (6.93)	0.8 (1.78)
Oleyl	Oleyl	C ₈₈ H ₁₆₁ N ₅ O ₈ -2.5H ₂ O	71.64 (71.82)	11.55 (11.63)	4.67 (4.87)	3.10 (3.13)
n-Dodecyl	H	C ₃₈ H ₇₃ N ₅ O ₈	62.56 (62.69)	9.64 (10.11)	9.67 (9.62)	0.27 (0.0)
Geranyl	H	C ₃₄ H ₅₇ N ₅ O ₈	61.30 (61.52)	8.71 (8.65)	10.67 (10.55)	2.71 (0.0)
Farnesyl	H	C ₄₄ H ₇₃ N ₅ O ₈ -2H ₂ O	62.94 (63.21)	8.87 (9.28)	8.21 (8.21)	3.25 (4.3)
Oleyl	C ₃ H ₇	C ₅₆ H ₁₀₅ N ₅ O ₈ -0.5 H ₂ O	68.02 (68.25)	10.76 (10.84)	6.91 (7.11)	0.67 (0.91)
Oleyl	C ₄ H ₉	C ₅₈ H ₁₀₉ N ₅ O ₈ -0.5 H ₂ O	68.51 (68.73)	11.06 (10.94)	6.85 (6.91)	0.32 (0.89)
Stearyl	C ₂ H ₅ O	C ₅₄ H ₁₀₁ N ₅ O ₁₀ -H ₂ O	65.28 (64.96)	11.00 (10.40)	6.19 (7.01)	4.81 (1.8)
Oleyl	Hexyl	C ₆₂ H ₁₁₇ N ₅ O ₈ -2HCl ^b	65.98 (65.69)	10.57 (10.57)	6.33 (6.33)	0.4 (0.0)
Eruyl	H	C ₅₈ H ₁₀₉ N ₅ O ₈ -0.5 H ₂ O	68.40 (68.73)	10.88 (10.94)	6.48 (6.91)	0.99 (0.88)
Petrosinyl	H	C ₅₀ H ₉₃ N ₅ O ₈ -H ₂ O	65.83 (65.98)	10.75 (10.52)	7.78 (7.69)	1.88 (1.97)
Ricinoleyl	H	C ₅₀ H ₉₃ N ₅ O ₁₀ -H ₂ O	64.10 (63.73)	10.16 (10.16)	7.53 (7.43)	1.60 (1.91)
Elaidyl	H	C ₅₀ H ₉₃ N ₅ O ₈	66.97 (67.30)	10.47 (10.51)	7.84 (7.85)	0.24 (0.0)
Linoleyl	H	C ₅₀ H ₈₉ N ₅ O ₈ -0.5 H ₂ O	67.06 (66.93)	10.25 (10.11)	7.87 (7.81)	0.9 (1.0)
Oleyl	OH	C ₅₀ H ₉₃ N ₅ O ₁₀ -H ₂ O	63.27 (63.37)	9.97 (10.16)	7.33 (7.43)	0.54 (1.91)

- a. Two different hydrates were isolated in this case.
 b. As a result of the HCl workup, the isolated product was isolated as an HCl salt.

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N-Mono-oleylamide of Diethylenetriaminepentaacetic Acid (DTPA-MOA)

Under a static nitrogen atmosphere, a mechanically stirred mixture of DTPA dianhydride (2.2-4.0 mole) and anhydrous pyridine (1.5-3.7 mole/mole of DTPA dianhydride) in anhydrous DMSO (1.5-1.7 L/mole of DTPA dianhydride) was heated to 70-85°C to dissolve most of the DTPA dianhydride. Neat oleylamine (1 mole) was then added dropwise to this solution at 60-65°C over a 5-15 minute period and the resulting mixture was held at 60-65°C for an additional hour. Water (18.6-24.9 mole) was added in one portion at 65-70°C and stirring continued for an additional hour. After cooling to room temperature, the mixture was diluted with chloroform to precipitate a tacky solid, which was filtered off with the aid of Celite. The red filtrate was concentrated in vacuum to remove the chloroform and most of the DMSO. The resulting residue was diluted with acetone to afford a tan solid, which was a mixture of the mono- and bis(amides) by TLC analysis [silica gel with 75:22:3 (V/V/V) CHCl₃:CH₃OH:H₂O]. These amides were readily separated by column chromatography on silica gel (100-200 mesh) using 75:22:3 (V/V/V) CHCl₃:CH₃OH:H₂O as eluant. The bisamide was isolated in 42-44% yield based on oleylamine. The mono(oleylamide) of DTPA was isolated as a white solid in 29-32% yield based on oleylamine after recrystallization from ethanol or methanol/ether mixtures in several crops. mp=205-8°C. This material was further characterized by high

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performance liquid chromatography, infrared spectroscopy,
proton and ¹³C nuclear magnetic resonance spectroscopy.

Anal. Calcd for C₃₂H₅₈N₄O₉-0.5H₂O: C, 58.97; H,
9.12%; N, 8.60; H₂O, 1.37%. Found: C, 59.06%; H, 9.22%;
N, 8.51%; H₂O, 1.35%.

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General Procedure for Gadolinium Complexes with DTPA Bis(amide) Ligands

A stoichiometric equivalent of gadolinium oxide and the corresponding DTPA Bis(amide) in 75:22:3 (V/V/V) CHCl₃:CH₃OH:H₂O (\approx 0.1 mole of complex/L of solvent) was heated at reflux with stirring for 18-25 hrs. After cooling to ambient temperature, the solution was filtered through Celite to remove trace Gd₂O₃. The filtrate was concentrated in vacuo to yield an off-white solid or glass. The resulting solid was recrystallized from appropriate solvent or slurried in hot acetone as shown in Table 3 unless noted otherwise. In some cases, the resulting solid was dried in a vacuum oven at 50-65°C and 29" Hg vacuum overnight. Successful complexation was evident by the dissolution of Gd₂O₃ into the organic solvent mix, thin layer chromatography (TLC) relative to the free ligand and infrared spectroscopy. In general, high performance liquid chromatography (HPLC) of the isolated products were greater than 90% one component. In the case of the unsymmetrically N,N-disubstituted DTPA amides, four major peaks were evident by TLC and HPLC analyses suggesting isomers due to the two achiral nitrogen atoms in the complexed ligand and restricted rotation around amide bonds.

Gadolinium Complex of Bis((N,N-diethyl)amide) of DTPA (GdDTPA-BDOA). Following the above procedure, resulting glass after concentration in vacuo was separated on a silica gel column with increasing ratios of methanol

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to chloroform. Two different fractions were combin d based on differences in 3000-3500 cm¹ infrared region. HPLC analyses indicated that these two fractions were the same. Elemental analyses showed different amounts of water and chloride for the two fractions suggesting that they were different hydrates containing varying amounts of chloroform and/or HCl and other contaminants as indicated below.

10	Fraction	Water (Wt%)	Cl (Wt%)	Carbon (Wt%)	Hydrogen (Wt%)	Nitrogen (Wt%)	Gd (Wt%)
	1	0.97	4.54	54.2	8.99	6.55	12.82
	2	1.80	9.61	48.01	8.05	6.01	12.99

15 Gadolinium Complex of Bis((N,N-dioleyl)amide) of DTPA (GdDTPA-BDO1A). The resulting glass after concentration in vacuo was separated on a silica gel column with 9:1 CHCl₃:CH₃OH to remove dioleylamine hydrochloride salt as an early eluting impurity. The desired product was isolated as a waxy solid after combining fractions and removal of the solvents on a rotary evaporator.

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Gadolinium Complex with DTPA Mono(oleylamide)
(GdDTPA-MOA). Under a static nitrogen atmosphere, a
magnetically stirred mixture of DTPA mono(oleylamide) (5.0
gm; 7.8 mmole) and gadolinium oxide (1.45 gm; 4 mmole) in
5 100 ml of 75:22:3 (V/V/V) CHCl₃:CH₃OH:H₂O was heated at
reflux for 25 hrs. The solution was suction filtered with
the aid of Celite to remove trace Gd₂O₃. The filtrate was
concentrated in vacuo to yield a beige solid. This solid
was slurried in acetone, suction filtered and dried in a
10 dessicator under vacuum; 6.1 gm (98% yield), mp>300°C.
This gadolinium complex was characterized further by
infrared spectroscopy and high performance liquid
chromatography.

Anal. Calcd for C₃₂H₅₅N₄O₉Gd-2.0 H₂O: C, 46.14;
15 H, 7.14%; N, 6.73; Gd, 18.88; H₂O, 4.32%. Found: C,
45.02%; H, 6.53%; N, 6.49%; Gd, 18.32; H₂O, 3.89%. The
measured ratio of carbon to gadolinium (2.457) was in
excellent agreement with the calculated ratio (2.444),
suggesting that the material was contaminated with
20 another element.

Table 3. Gd-DTPA Bisamide Complexes

Compound	R ₁	R ₂	Recryst Solvent	Yield	mp (°C)
GdDTPA-BOA	Oleyl	H	CHCl ₃ /MeOH/Acetone	85%	>300
GdDTPA-BDOA	Oleyl	Oleyl	>100% ^a	95%	glass
GdDTPA-BMSA	Stearyl	Methyl	Acetone ^b	99%	276-82
GdDTPA-BHDPA	4-n-Hexadecyl-phenyl	H	Acetone	>100% ^a	>275
GdDTPA-BDOA	Oleyl	Oleyl	Acetone ^c	72%	waxy solid
GdDTPA-BDDA	Dodecyl	H	EtOH/Acetone	80%	>300
GdDTPA-BGA	Geranyl	H	Acetone	97%	>300
GdDTPA-BFA	Farnesyl	H	Acetone	>100% ^a	d
GdDTPA-BSA	Stearyl	H	Acetone	100%	d
GdDTPA-BHOA	Oleyl	Hexyl	None	93%	d
GdDTPA-BRA	Ritinoleyl	H	CHCl ₃ /MeOH/Acetone	94	330-334 (dec)
GdDTPA-BPA	Petroselinyl	H	CHCl ₃ /MeOH/Acetone	94	333-335 (dec)
GdDTPA-BERA	Erucyl	H	CHCl ₃ /MeOH/Acetone	99%	332-334 (dec)
GdDTPA-BEA	Elaidyl	H	CHCl ₃ /MeOH/Acetone	98%	330-332 (dec)
GdDTPA-BLA	Linoleyl	H	CHCl ₃ /MeOH/Acetone	95%	>360
GdDTPA-BPOA	Oleyl	C ₃ H ₇	CHCl ₃ /MeOH/Acetone	90%	160 (dec)
GdDTPA-BBuOA	Oleyl	C ₄ H ₉	Chromatography	60%	140 (dec)
GdDTPA-BHESA	Stearyl	C ₂ H ₅ O	Chromatography	96%	198-200 (dec)
GdDTPA-BEOA	Oleyl	C ₂ H ₅	Acetone ^b	99%	>245
GdDTPA-BBOA	Oleyl	C ₇ H ₇	none	98%	>270
GdDTPA-BHOA	Oleyl	OH	Chromatography	63%	245 (dec)

a. The crude product was obviously contaminated with solvent.
 b. The crude product was dissolved in hot acetone and continuously stirred with a magnetic stirrer while cooling to room temperature to yield a white solid.

c. The resulting solid isolated after column chromatography on silica gel was slurried in acetone at room temperature and filtered.
 d. Not determined

Table 4. Elemental Analyses of Gadolinium DTPA Bis-Amide Complexes

Compound	R ₁	R ₂	Molecular Formula	C	H	N	Gd	Water
GdDTPA-BOA	Oleyl	H	C ₅₀ H ₉₀ N ₅ O ₈ Gd-H ₂ O	56.17 (56.41)	8.51 (8.71)	6.33 (6.58)	15.44 (14.77)	0.21 (1.69)
GdDTPA-BMSA	Stearyl	Methyl	C ₅₂ H ₉₈ N ₅ O ₈ Gd-2H ₂ O	55.81 (56.03)	9.16 (9.22)	5.98 (6.28)	14.44 (14.11)	2.96 (3.23)
GdDTPA-BHDP	4-n-Hexa-decyphenyl	H	C ₅₈ H ₉₄ N ₅ O ₈ Gd-1.5H ₂ O	59.18 (59.36)	8.39 (8.33)	5.82 (5.97)	13.67 (13.40)	2.51 (2.30)
GdDTPA-BDOIA	Oleyl	Oleyl	C ₈₆ H ₁₅₈ N ₅ O ₈ Gd-0.5H ₂ O	66.02 (66.36)	10.22 (10.3)	4.19 (4.5)	10.09 (10.1)	0.47 (0.58)
GdDTPA-BDDA	Dodecyl	H	C ₃₈ H ₇₀ N ₅ O ₈ Gd-0.5H ₂ O	51.27 (51.73)	8.13 (8.0)	8.06 (7.94)	16.83 (17.82)	0.83 (1.0)
GdDTPA-BGA	Geranyl	H	C ₃₄ H ₅₄ N ₅ O ₈ Gd-0.5H ₂ O	49.09 (49.38)	6.57 (6.57)	8.32 (8.32)	19.68 (19.01)	0.8 (1.08)
GdDTPA-BFA	Farnesyl	H	C ₄₄ H ₇₀ N ₅ O ₈ Gd-H ₂ O	52.48 (54.35)	7.32 (7.46)	7.18 (7.20)	13.19 (16.17)	1.6 (1.85)
GdDTPA-BSA	Stearyl	H	C ₅₀ H ₉₄ N ₅ O ₈ Gd-2H ₂ O	55.61 (55.26)	8.99 (9.09)	6.47 (6.44)	14.77 (14.47)	3.90 (3.32)
GdDTPA-BHOA	Oleyl	Hexyl	C ₆₂ H ₁₁₄ N ₅ O ₈ Gd-4H ₂ O	57.87 (57.86)	9.17 (9.56)	5.37 (5.44)	12.62 (12.22)	2.73 (5.60)
GdDTPA-BPA	Petroselinyl	H	C ₅₆ H ₉₀ N ₅ O ₈ Gd-0.5H ₂ O	56.60 (56.90)	8.54 (8.69)	6.66 (6.63)	15.46 (14.90)	0.40 (0.85)
GdDTPA-BRA	Ricinoleyl	H	C ₅₀ H ₉₀ N ₅ O ₁₀ Gd-0.5H ₂ O	54.90 (55.22)	8.52 (8.43)	6.53 (6.44)	14.87 (14.46)	0.58 (0.82)
GdDTPA-BERA	Erucyl	H	C ₅₈ H ₁₀₆ N ₅ O ₈ Gd-4.5H ₂ O	55.83 (56.18)	9.23 (9.35)	5.32 (5.65)	13.34 (12.68)	6.25 (6.54)
GdDTPA-BLA	Linoleyl	H	C ₅₀ H ₈₈ N ₅ O ₈ Gd-H ₂ O	56.17 (56.63)	8.28 (8.26)	6.63 (6.6)	15.11 (14.83)	1.45 (1.69)
GdDTPA-BEA	Elaidyl	H	C ₅₀ H ₉₀ N ₅ O ₈ Gd-0.5H ₂ O	56.55 (56.90)	8.75 (8.69)	6.24 (6.63)	15.06 (14.9)	0.72 (0.85)
GdDTPA-BPOA	Oleyl	C ₃ H ₇	C ₅₆ H ₁₀₂ N ₅ O ₈ Gd-1.5H ₂ O	58.05 (58.10)	9.09 (9.14)	5.87 (6.05)	6.56 (13.58)	2.16 (2.33)
GdDTPA-BBuOA	Oleyl	C ₄ H ₉	C ₅₈ H ₁₀₆ N ₅ O ₈ Gd -1H ₂ O-0.5 HCl	58.04 (57.86)	9.19 (9.17)	5.57 (5.82)	6.30 (13.06)	2.17 (2.24)
GdDTPA-BHESA	Stearyl	C ₂ H ₅ O	C ₅₄ H ₁₀₁ N ₅ O ₁₀ Gd -3.75 H ₂ O-0.75 HCl	54.74 (54.88)	8.93 (9.07)	5.41 (5.71)	12.35 (12.83)	5.34 (5.51)
GdDTPA-BEOA	Oleyl	C ₂ H ₅	C ₅₄ H ₉₈ N ₅ O ₈ Gd-H ₂ O	57.76 (57.88)	9.09 (8.99)	6.16 (6.25)	13.60 (14.03)	1.52 (1.61)
GdDTPA-BBOA	Oleyl	C ₇ H ₇	C ₄₂ H ₇₆ N ₅ O ₈ Gd-H ₂ O	52.43 (52.86)	8.43 (8.24)	7.18 (7.34)	17.14 (16.48)	1.97 (1.89)
GdDTPA-BOHA	Oleyl	OH	C ₅₀ H ₉₀ N ₅ O ₁₀ Gd-H ₂ O	54.51 (54.77)	8.57 (8.46)	6.41 (6.39)	14.37 (14.34)	1.58 (1.64)

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Preparations of GdDTPA Bis(oleylamide) Emulsions

All GdDTPA Bis(oleylamide) experimental emulsions in Table 5 were prepared in 28 mL volumes using an M-110S microfluidizer. The equipment was flushed with deionized water between each emulsion formulation. The emulsion components: Water, safflower oil, PFDCO (perfluorodichlorooctane), gadolinium complex and lecithin were weighed into a 30 mL capacity blender cup. The water component contained approximately 300 μ l of 5% NaCO₃ to adjust the material pH to a physiological range. On average, emulsions showed a 1.5 pH drop during sterilization. The mix was blended for 30-60 seconds depending on the concentration of water in the sample. The sample was transferred to the reservoir of the microfluidizer and emulsified for three minutes. To prevent the emulsions from heating excessively during homogenation, the shear valve and mixing coil were immersed in a room temperature water bath during processing. The final temperature of the emulsion samples was approximately 35°C. Completed emulsions were bottled in 30mL serum vials, with a head of nitrogen gas. Autoclave conditions were: 4-7 minutes heat up to sterilization temperature, sterilization at 121°C for 15 minutes and a 15 minute pressurized cold water cool down to final temperatures at 25-30°C.

In general, with reference to Table 5, the quality of the emulsions was excellent, with the exceptions noted. Microscopically, emulsions containing

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no oil component showed incomplete dispersion of the GdDTPA-Bis(amide) complex. Emulsions exhibited high viscosity (or a gel like consistency) when either the safflower oil or the PFC content was greater than about 5 25% w/v or about 55 v/v%, respectively. The gadolinium complex exhibited surfactant activity during emulsification, except foaming occurred. Upon separation from the foam, a high quality stable emulsion was isolated. Therefore, a separate surfactant may be 10 eliminated in certain cases where a chelate complex exhibits sufficient surfactant activity.

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Table 5. Gadolinium DTPA-BOA Emulsions

Emulsion	Lec.	Oil	PFC	Gd	pH Post	pH Osm	Osm Post	Visc Post	PSD Post	CM Post	CV Post
1	2.0%	9.9%	0.0%	4.9%	8.07	6.80	318	320	1.74	1.80	158
2	2.0%	25.0%	0.0%	5.1%	8.08	6.60	312	308	3.45	3.96	127
3	2.0%	49.5%	0.0%	5.0%	8.30	7.01	326	296	-	140	143
4	0.5%	9.8%	0.0%	5.0%	8.16	7.08	303	300	1.51	1.74	182
5	0.5%	23.9%	0.0%	5.0%	8.30	6.77	356	357	3.71	3.77	134
6	9.8%	24.6%	0.0%	5.2%	8.47	7.10	379	262	5.74	20.50	101
7	2.0%	10.1%	0.0%	2.0%	8.10	7.21	340	344	1.10	1.39	180
8	2.0%	10.0%	0.0%	5.0%	8.35	7.19	306	298	1.51	1.64	186
9	2.0%	9.8%	0.0%	9.9%	8.48	7.22	324	323	2.59	3.47	155
10	2.0%	0.0%	9.9%	5.0%	8.03	6.57	322	315	1.31	1.45	39
11	2.0%	0.0%	24.8%	5.0%	8.39	6.97	367	377	3.26	3.51	134
12	2.0%	0.0%	39.7%	5.0%	8.73	7.32	396	296	18.70	10.10	111
13	0.5%	0.0%	24.4%	4.9%	9.02	7.18	661	666	3.41	3.12	117
14	10.1%	0.0%	25.0%	5.0%	8.43	7.22	389	393	6.35	6.71	74
15	2.0%	0.0%	9.6%	2.1%	8.61	7.47	593	585	1.29	1.03	82
16	2.0%	0.0%	10.4%	10.0%	8.53	7.10	485	483	2.02	2.20	73
17	2.0%	1.9%	10.0%	2.0%	7.80	6.72	318	281	1.33	1.39	133
18	2.0%	2.0%	9.8%	5.0%	7.95	6.65	311	324	1.41	1.61	171
19	2.1%	9.1%	1.2%	5.1%	8.66	7.35	353	329	1.51	2.22	14
20	3.0%	10.2%	39.8%	5.0%	8.79	7.50	254	126	-	-	99
21	3.0%	39.7%	10.2%	5.0%	8.69	7.40	156	214	-	-	119
22	2.1%	10.1%	0.0%	5.0%	8.74	7.17	297	295	1.79	1.84	109
23	2.1%	2.1%	10.1%	5.2%	8.75	7.26	364	379	1.99	2.59	84
24	2.0%	0.0%	11.8%	4.9%	8.94	7.46	307	297	1.69	1.77	99
25	2.0%	9.9%	0.0%	5.0%	8.33	6.73	327	328	2.62	2.56	106
26	2.0%	1.9%	10.0%	5.0%	10.27	7.70	324	324	2.44	2.45	200
27	2.0%	9.9%	0.0%	5.0%	8.33	6.73	327	328	2.62	2.56	106

Visc = viscosity in centipoise (cP)

CM = mean particle size above 0.78 μm

Osm = osmolarity (mOs/kg)

PSD = submicron mean particle size (nm)

CV = volume percent of particles above 0.78 μm

post = post sterilization values (121°C for 15 minutes)

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With reference to the above Table 5, Examples 3 and 21 demonstrated a gelatinous consistency; and Examples 6, 10 and 17 separated into two layers upon storage. With respect to Examples 22, 23 and 24, the surfactant was 1,3-dihexyldecylglycero-2-phosphoryl choline. Furthermore, the surfactant employed in Examples 25, 26 and 27 was Pluronic F68 and, with respect to Example 27, it was of a pasty consistency.

Using the above general procedure for making the GdDTPA bis(oleylamide) emulsions, other Gd or Mn chelate complex emulsions were made with oil and/or PFC dispersed oil phases and with various ligands for the NR₁R₂ group of the above general formula. The key to the acronyms for the ligands are shown in Table 6.

With reference to Table 6, very fine and stable emulsions were obtained for complexes having an unsaturated group with a single, double or plurality (2 or greater) of double bond(s) and at different locations in the group. Branched or straight chain groups are also suitable.

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Table 6. Key to Ligands

BOA = bis(oleylamide)	BMSA = bis(N-methyl-N-stearylamine)
BHEOA = bis(N-hydroxyethyl-N-oleylamide)	MOA = monooleylamide
BSHA = bis(N-stearyl-N-hydroxylamine)	BHESA = bis(N-hydroxyethyl-N-stearylamine)
BEOA = bis(N-ethyl-N-oleylamide)	BBOA = bis(benzoyloleylamide)
BHOA = bis(N-hexyl-N-oleylamide)	BSA = bis(stearylamine)
BGA = bis(geranylamine)	BBuOA = bis(N-buty-N-oleylamide)
BFA = bis(farnesylamine)	BPOA = bis(N-propyl-N-oleylamide)
BHDPA = bis(N-hexadecylphenylamide)	BERA = bis(eucetylamine)
BOHA = bis(N-oleyl-N-hydroxylamine)	BDDA = bis(dodecylamine)
BEA = bis(elaidylamide)	BHDA = bis(hexadecylamide)
BTDA = bis(tetradecylamide)	BLA = bis(linoleylamide)
BRA = bis(ricinoleylamine)	BDOA = bis((N,N-diacyl)amide)
BDOLA = bis((N,N-diacyl)amide)	BODP = bis(N-octadecyl-N-propanediolamide)
BPA = bis(petroselinyl)	

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The MRI emulsions of this invention that have been made in accordance with the above detailed description were characterized into various categories of emulsions for comparison with other paramagnetic metal ion chelate complexes outside the scope of this invention.

5 According to this invention, the paramagnetic metal chelate complexes that make fine emulsions have been categorized as those in accordance with the above general formula where A.) R₁ is a single straight or branched long carbon chain (C₁₀-C₃₀) on the nitrogen that is unsaturated (or has multiple unsaturations), or B.) R₁ is a long straight or branched carbon chain (C₁₀-C₃₀) on the nitrogen that is unsaturated (or has multiple unsaturations) and R₂ is a short chain (C₁-C₂) and C.) R₁ is a long straight or

10 branched carbon chain (C₁₀-C₃₀) on the nitrogen that is unsaturated (or has multiple unsaturations) and has a hydroxyl group on the nitrogen. The categories of emulsions and complexes of this invention that make fine emulsions are compared to other emulsions and complexes

15 that make poor emulsions as reported in Table 7.

20

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Table 7. Categories of Emulsions and Complexes

	R ₁	R ₂	Category #
5	Poor (comparative)		
	C _{≥10} - Saturated	H	1
	C _{≥10} - Saturated	C _{≥1} - Saturated	2
10	C _{≥10} - Unsaturated	C _{≤2} Sat./Unsat.	3
	Fine (invention)		
	C _{≥10} - Unsaturated	H	4
	C _{≥10} - Unsaturated	C _{≤2} Alkyl	5
	C _{≥10} - Unsaturated	OH	6

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As may be determined from the above Table 7,
the emulsions and chelate complexes of categories 4-6 are
representative of those MRI chelate complexes that make
fine emulsions in accord with this invention. Those
5 emulsions and chelate complexes of categories 1-3 make
poor emulsions. The tabular summaries for each of the
above categories 1-6 are reported in the following Tables
8-13.

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Table 8. Comparative Category #1 (single saturated chain)

Active Component	% Iec./% oil	Quality	R1	R2	Visc	PSD	CM	CV
5% GdDTPA-BSA	2/10	Solids	C18H37	H	35.70	194	6.54	48.4
5% GdDIPA-BSA	2/5	Solids	C18H37	H	*	212	8.28	98.8
5% GdDIPA-BSA	2/20	Solids	C18H37	H	*	191	7.11	72.8
5% GdDTPA-BSA	4/10	Solids	C18H37	H	9.44	194	7.73	82.4
5% GdDIPA-BSA	4/5	Solids	C18H37	H	9.77	188	7.83	86.6
5% GdDTPA-BSA	4/20	Solids	C18H37	H	9.52	269	5.85	32.9
5% GdDTPA-BDDA	2/10	Solids	C12H25	H	1.8	210	2.34	19.8
5% GdDTPA-BHDDA	2/10	Solids	C16H33	H	2.59	184	3.55	39.8
5% GdDTPA-BTDA	2/10	Solids	C14H29	H	1.55	196	2.73	18.24
5% GdDTPA-BHDDA	2/10	Solids	C22H37	H	2.82	195	6.63	100

FOOTNOTE FOR TABLES 8-13

Quality = as viewed at 1200X

Visc. = viscosity in centipose (cP)

CM = mean particle size above 0.78 μ m

PSD = submicron mean particle size (nm)
 CV = volume percent of particles above 0.78 μ m

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Table 9. Comparative Category #2 (two saturated chains)

Active Component	% Icc./% oil	Quality	R1	R2	Visc	PSD	CM	CV
5% GdDTPA-BMSA	2/10	Solids	C18H37	CH3	2.04	532	2.41	12.52
5% MnEDTA-BODP	2/10	Solids	C18H37	C3H7O2	2.87	187	4.139	35.40
5% GdDTPA-BDOA	2/10	Failed	C8H17	C8H17	--	--	--	--

Table 10. Comparative Category #3 (one unsaturated chain, one long chain C>2)

Active Component	% Icc./% oil	Quality	R1	R2	Visc	PSD	CM	CV
5% GdDTPA-BDOA	2/10	Failed	C18H35	C18H35	--	--	--	--
5% GdDTPA-BBOA	2/10	Failed	C18H35	C7H7	--	--	--	--
5% GdDTPA-BPOA	2/10	Solids	C18H35	C3H7	24.4	569	7.11	43.1
5% GdDTPA-BBuOA	2/10	Solids	C18H35	C4H9	2.17	*	9.12	54.4
5% GdDTPA-BHOA	2/10	Failed	C18H35	C6H13	--	--	--	--

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Table 11. Invention Category #4 (one unsaturated chain)

Active Component	% Iec./% oil	Quality	RI	R2	Visc	PSD	CM	CV
5% GdDTPA-BOA	2/10	Excellent	C18H35	H	1.64	184	0.82	0.44
2% GdDTPA-BOA	2/10	Excellent	C18H35	H	1.39	179	0.82	0.44
10% GdDTPA-BOA	2/10	Excellent	C18H35	H	3.47	161	<0.73	0.40
5% GdDTPA-BOA	0.5/10	Excellent	C18H35	H	1.74	185	<0.68	0.80
5% GdDTPA-MOA**	2/10	Excellent	C18H35	H	1.66	134	0.852	0.88
5% GdDTPA-BFA	2/10	Excellent	C15H25	H	1.70	167	1.31	0.32
5% GdDTPA-BGA	2/10	Very Good	C10H17	H	1.35	185	1.29	1.92
5% GdDTPA-BEA	2/10	Very Good	C22H43	H	2.29	162	1.29	0.32
5% GdDTPA-BEA	2/10	Very Good	C18H35	H	3.12	142	2.04	4.68
5% GdDTPA-BLA	2/10	Excellent	C18H33	H	1.67	116	1.01	0.28
5% GdDTPA-BPA	2/10	Very Good	C18H35	H	1.54	124	0.83	0.24
5% GdDTPA-BRA	2/10	Good	C18H35O	H	4.43	108	1.4	2.68

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Table 12. Invention Category #5 (one unsaturated chain, one alkyl C_{≤2})

Active Component	% Iec./% oil	Quality	R ₁	R ₂	Visc	PSD	CM	CV
5% GdDTPA-BEOA	2/10	Excellent	C ₁₈ H ₃₅	C ₂ H ₅	1.87	206	0.88	0.36

Table 13. Invention Category #6 (one -OH, and one unsaturated chain C_{≥10})

Active Component	% Iec./% oil	Quality	R ₁	R ₂	Visc	PSD	CM	CV
5% GdDTPA-BOHA	2/10	Excellent	C ₁₈ H ₃₅	OH	2.62	165	1.51	0.32

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With respect to Comparative Category #1 as represented by Table 8, it was found that where R₁ was a single saturated chain and R₂ was hydrogen as represented by GdDTPA-BSA, poor emulsions were made despite a variety 5 of compositions containing a surfactant lecithin, oil and PFDCO. Furthermore, with respect to those emulsions indicated by an asterisk(*), they were too viscous to measure. With reference to Comparative Categories #2 and #3 in Tables 9 and 10, where R₁ and R₂ were two saturated 10 chains or one unsaturated chain and one long chain C₁-C₂, the emulsions had huge solids or simply failed to emulsify. In Table 10, the asterisk (*) indicates that the emulsion cracked upon sterilization and the particle sizes were too large to be measured by sub-micron particle 15 sizer.

In contrast, the invention is represented by Tables 11, 12 and 13 where Categories Nos. 4, 5 and 6 are shown. In other words, where R₁ was a C₁₀-C₃₀ unsaturated chain and R₂ was either hydrogen, hydroxyl or a C₁-C₂ 20 alkyl, excellent emulsions were obtained.

Furthermore, at the present time, the most preferred paramagnetic metal chelate complex is GdDTPA-BOA which provides for an excellent emulsion with CVs above 0.8 micron being less than 1%. In addition, the monoamide version of GdDTPA-BOA also provided an excellent emulsion. 25 With reference to Table 5, a summary of the many formulations is provided for the most preferred compound

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GdDTPA-BOA having different compositions of oil alone, or combinations of oil and PFDCO, or PFDCO alone. For the most part, all of the emulsions in Table 5 were excellent with CVs above 0.8 micron again less than 1%, with the
5 noted exceptions.

With respect to Tables 14, 15 and 16, the stabilities of the emulsions made with GdDTPA-BOA at 40°C (accelerated), 0°C (refrigerated) and 25°C (room temperature) were determined. As may be determined with
10 respect to these tables, emulsions of this invention provided excellent stability over the period of time indicated. However, those emulsions that were noted above to be poor emulsions typically become a gel (solid mass) by three months in refrigeration and thus no data can be measured for these. Also, the photographs of Figure 1
15 at 400 X magnification are representative of the "fine" and "poor" emulsions. At a magnification of 400 x, a 10 micron particle will be about 4mm in the photo.

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Table 14. 40°C Stability Data for GdDTPA-BOA

2% Lecithin/10% oil/5% Complex	0 Time	2 Weeks	4 Weeks	6 Weeks
GdDTPA-BOA by HPLC (% w/w)	4.85	4.70	4.70	5.13
pH	6.78	6.53	7.14	6.47
Viscosity (cP)	1.87	1.84	2.02	1.87
Submicron mean particle size (nm)	165	167	167	169
Mean particle size above 0.78 µm (µm)	0.93	0.63	1.09	0.81
Volume percent of particles above 0.78 µm	0.16	0.56	0.32	0.56
Visual inspection	normal	normal	normal	normal
Microscopic exam	excellent	excellent	excellent	excellent

Table 15. 4°C Stability Data for GdDTPA-BOA

2% Lecithin/10% oil/5% Complex	0 Time	3 Months	6 Months
GdDTPA-BOA by HPLC (% w/w)	4.85	5.36	5.89
pH	6.78	6.95	6.73
Viscosity (cP)	1.87	2.07	2.15
Submicron mean particle size (nm)	165	170	169
Mean particle size above 0.78 µm (µm)	0.93	1.05	0.99
Volume percent of particles above 0.78 µm	0.16	0.52	0.16
Visual inspection	normal	normal	excellent
Microscopic exam	excellent	excellent	excellent

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Table 16. 25°C Stability Data for GdDTPA-BOA

2% Lecithin/10% oil/5% Complex	0 Time	3 Months	6 Months
GdDTPA-BOA by HPLC (% w/w)	4.85	5.31	5.96
pH	6.78	6.81	6.61
Viscosity (cP)	1.87	2.15	1.87
Submicron mean particle size (nm)	165	169	166
Mean particle size above 0.78 µm (µm)	0.93	0.84	0.93
Volume percent of particles above 0.78 µm	0.16	0.72	0.12
Visual inspection	normal	normal	normal
Microscopic exam	excellent	excellent	excellent

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The MRI utilities of the emulsions have been determined by using an emulsion containing 5% of GdDTPA-BOA to improve 3D time - of - flight angiography in a rabbit. Figure 1A shows a number of major cerebral arteries and branches that can be easily visualized in the post-contrast image that could not be appreciated before administration of the chelate complex. Angiography is often difficult to perform in the liver and Figure 1B shows that little or no vascular detail can be seen in the pre-contrast liver, whereas with the post-contrast GdDPTA-BOA emulsion of this invention, visualization of vascular structures is allowed within this organ. Thus, with reference to both the brain and the liver, a 10 μ mole of gadolinium per kg dose was administered intravenously and the enhancement effect on the angiograms persisted for at least one hour. Both images were collected with the standard 3D time - of - flight imaging sequence on a General Electric Signa whole body clinical scanner operating at 1.5 Tesla.

An emulsion containing 4.2% of GdDPTA-MOA was used to enhance the liver of a rabbit. Figure 2 shows that nearly 150% enhancement occurs in the liver after intravenous administration of a 10 μ mole of gadolinium per/kg dose. The enhancement effect on the liver persisted for at least one hour. The images were collected with a standard T₁-weighted spin-echo imaging sequence on a General Electric Signa whole body clinical scanner operating at 1.5 Tesla.

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For comparison with the emulsions of this invention, Examples I and VII were reproduced from U.S. Patent 5,120,527, mentioned in the above background of this invention. Although the emulsions made under this 5 '527 patent according to these procedures do not have visible solids, they have very large particles on the order of 10 to about 30 microns and hence are unacceptable for IV use. With respect to the emulsion containing Geritol®, the composition contained 60mL Geritol®, 150mL melted ice cream, 250mL milk and 100mL corn oil; with other properties including viscosity of 6.05 cp, CM of 10 8.17 microns and CV of 83.1%. The Geritol® emulsion also cracked upon sterilization. The GdDTPA emulsion contained 0.5mole (1.0mL) of GdDTPA, 150mL melted ice cream, 250mL 15 milk and 100mL corn oil; with the other properties of the emulsion including a viscosity with 8.18cp, CM of 16.3 microns and CV of 71.8%. In summary, the emulsions of the '527 patent are unacceptable for IV use and do not have the versatility of the emulsions of this invention. They 20 also lack stability upon sterilization as evidenced by the above experiments.

In view of the above detailed description, other variations or modifications may be made without departing from the spirit and scope of this invention.

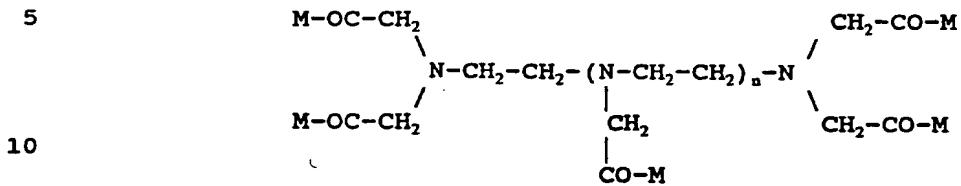
25 What is claimed is:

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CLAIMS

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1. A physiologically acceptable emulsion for enhancement of MRI imaging comprising water, a dispersed oil phase and a paramagnetic metal complex having a C₁₀-C₃₀ unsaturated aliphatic group.
 2. A physiologically acceptable emulsion for enhancement of MRI imaging comprising water, a dispersed oil phase and a complex of a paramagnetic metal ion and an organic chelator having the formula



wherein from 2 to 5 M groups are hydroxyl, n=0 to 2, and any remaining M group is NR₁R₂, each R₁ is a C₁₀-C₃₀ unsaturated aliphatic group and R₂ is hydrogen, hydroxyl or a C₁-C₂ alkyl.

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3. The physiologically acceptable emulsion of claim 2 wherein said metal ion is a lanthanide element of atomic numbers 58-70 or a transition metal of atomic numbers 21-29, 42 or 44.

4. The physiologically acceptable emulsion of claim 2 wherein said metal ion is selected from a group consisting Gd(III), Mn(II), iron and dysprosium.

5. The physiologically acceptable emulsion of claim 2 wherein said organic chelator is an acid selected from the group consisting of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid.

6. The physiologically acceptable emulsion of claim 2 wherein said organic chelator is a monoamide or a bisamide of an organic acid selected from a group consisting of diethylenetriaminepentaacetic acid and 5 ethylenediaminetetraacetic acid.

7. The physiologically acceptable emulsion of claim 2 wherein each R₁ is C₁₄-C₂₂.

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8. The physiologically acceptable emulsion of claim 2 wherein the organic chelator is a mono or bisamide where R₁ is selected from the group consisting of oleyl, farnesyl, geranyl, erucyl, elaidyl, linoleyl, ricinoleyl, 5 petroselinyl, linolenyl, vaccenyl, linderyl, palmitoleyl, palmitelaidyl, myristoleyl, and myristelaidyl, and where R₂ is selected from the group consisting of methyl, ethyl, and hydroxyl.
9. The physiologically acceptable emulsion of claim 2 wherein said complex is gadolinium diethylenetriaminepentaacetic acid bis(oleylamide).
10. The physiologically acceptable emulsion of claim 2 wherein said chelate complex is gadolinium diethylenetriaminepentaacetic acid mono(oleylamide).
11. The emulsion of claim 2 that is stable after heat sterilization with less than 10 volume % of particles above about 0.8 micron.
12. The emulsion of claim 2 wherein the oil phase contains about 5 to about 25% w/v oil or about 5 to about 55% v/v fluorochemical.
13. The emulsion of claim 2 wherein the oil is selected from the group consisting of mono-, di- and triglycerides, and mixtures thereof.

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14. The emulsion of claim 12 wherein the surfactant is present in an amount from about 0.5 to about 10% by weight of the emulsion.

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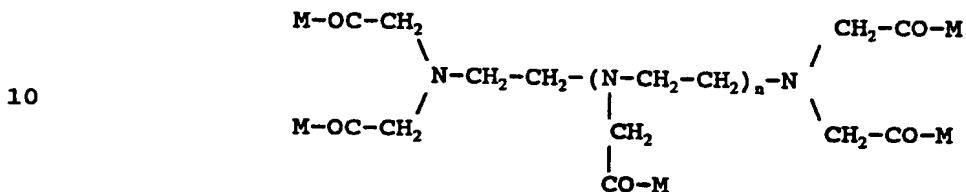
15. The physiologically acceptable emulsion of claim
2 wherein the chelate complex is selected from the group
consisting of gadolinium diethylenetriaminepentaacetic
acid bis(oleylamide), gadolinium
5 diethylenetriaminepentaacetic acid mono(oleylamide),
gadolinium diethylenetriaminepentaacetic acid
bis(farnesylamide), gadolinium diethylenetriaminepentaacetic acid
10 bis(geranylamide), gadolinium diethylenetriaminepentaacetic acid
bis(erucylamide), gadolinium diethylenetriaminepentaacetic
acid bis(elaidylamide), gadolinium diethylenetriaminepentaacetic acid
15 bis(linoleylamide), gadolinium diethylenetriaminepentaacetic acid
bis(petroselinyl), gadolinium diethylenetriaminepentaacetic acid
bis(linolenylamide), gadolinium diethylenetriaminepentaacetic acid
20 bis(vaccenylamide), gadolinium diethylenetriaminepentaacetic acid
bis(linderylamine), gadolinium diethylenetriaminepentaacetic acid
bis(palmitoleylamide), gadolinium diethylenetriaminepentaacetic acid
25 bis(palmitelaidylamide), gadolinium diethylenetriaminepentaacetic acid
bis(myristoleylamide), gadolinium diethylenetriaminepentaacetic acid
bis(myristelaidylamide), and the N-methyl, N-ethyl, and N-
OH derivatives of said gadolinium complexes.

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16. A physiologically acceptable emulsion for intravenous administration and enhancement of MRI imaging comprising water, a dispersed oil phase and a paramagnetic metal complex having a C₁₀-C₃₀ unsaturated aliphatic group, said emulsion having an average particle size less than about 1 micron.

17. The physiologically acceptable emulsion of claim
16 having an average particle size on the order of about
0.2 to about 0.4 micron.

18. A physiologically acceptable emulsion for enhancement of MRI imaging comprising water, a dispersed oil phase selected from the group consisting of an oil and fluoroochemical, and mixtures thereof, a surfactant, and a complex of a paramagnetic metal ion and an organic chelator having the formula



wherein from 2 to 5 M groups are hydroxyl, n=0 to 2 and
any remaining M group is NR₁R₂, each R₁ is a C₁₀-C₃₀
unsaturated aliphatic group and R₂ is hydrogen, hydroxyl
or a C₁-C₆ alkyl.

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19. The physiologically acceptable emulsion of claim 18 wherein said metal ion is a lanthanide element of atomic numbers 58-70 or a transition metal of atomic numbers 21-29, 42 or 44.
20. The physiologically acceptable emulsion of claim 18 wherein said metal ion is selected from a group consisting Gd(III), Mn(II), iron and dysprosium.
21. The physiologically acceptable emulsion of claim 18 wherein said organic chelator is an acid selected from the group consisting of ethylenediaminetetraacetic acid and diethylenetriaminepentaacetic acid.
22. The physiologically acceptable emulsion of claim 18 wherein said organic chelator is a monoamide or a bisamide of an organic acid selected from a group consisting of diethylenetriaminepentaacetic acid and ethylenediaminetetraacetic acid.
23. The physiologically acceptable emulsion of claim 18 wherein each R₁ is C₁₄-C₂₂.

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24. The physiologically acceptable emulsion of claim 18 wherein the organic chelator is a mono or bisamide where R₁ is selected from the group consisting of oleyl, farnesyl, geranyl, erucyl, elaidyl, linoleyl, ricinoleyl, petroselinyl, linolenyl, vaccenyl, linderyl, palmitoleyl, palmitelaidyl, myristoleyl, and myristelaidyl, and where R₂ is selected from the group consisting of methyl, ethyl, and hydroxyl.
- 5
25. The physiologically acceptable emulsion of claim 18 wherein said complex is gadolinium diethylenetriaminepentaacetic acid bis(oleylamide).
26. The physiologically acceptable emulsion of claim 18 wherein said chelate complex is gadolinium diethylenetriaminepentaacetic acid mono(oleylamide).

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27. The emulsion of claim 18 wherein the fluoroochemical is selected from the group consisting of perfluorodecalin, perfluoromethyldecalin, perfluorodimethyladamantane, perfluoroctylbromide, 5 perfluoro-4-methyloctahydroquinolidizine, perfluoro-N-methyl-decahydroquinoline, F-methyl-1-oxadecalin, perfluorobicyclo (5.3.0.) decane, perfluoroctahydroquinolidizine, perfluoro 5,6-dihydro-5-decene, perfluoro-4,5-dihydro-4-octene, 10 perfluorodichlorooctane, perfluorobischlorobutyl ether, and chlorinated perfluorocarbons, and mixtures thereof.
28. The emulsion of claim 18 that is stable after heat sterilization with less than 10 volume % of particles above about 0.8 micron.
29. The emulsion of claim 18 wherein the oil phase contains about 5 to about 25% w/v oil or about 5 to about 55% v/v fluoroochemical.
30. The emulsion of claim 18 wherein the oil is selected from the group consisting of mono-, di- and triglycerides, and mixtures thereof.

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31. The emulsion of claim 29 wherein the surfactant is present in an amount from about 0.5 to about 10% by weight of the emulsion.
32. The method for MRI imaging of a subject comprising administering to such subject an image-modifying effective amount of the emulsion of claim 1.
33. The method for MRI imaging of a subject comprising administering to such subject an image-modifying effective amount of the emulsion of claim 2.
34. The method for MRI imaging of a subject comprising administering to such subject an image-modifying effective amount of the emulsion of claim 15.
35. The method for MRI imaging of a subject comprising intravenously administering to such subject an image-modifying effective amount of the emulsion of claim 16.
36. The method for MRI imaging of a subject comprising administering to such subject an image-modifying effective amount of the emulsion of claim 18.
37. The method for MRI imaging of a subject comprising administering to such subject an image-modifying effective amount of the emulsion of claim 24.

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38. The method for MRI imaging of a subject comprising administering to such subject an image-modifying effective amount of the emulsion of claim 27.

39. The method for MRI imaging of a subject comprising intravenously administering to such subject an image-modifying effective amount of the emulsion of claim 28.

Figure 1. 3D TOF Angiography with GdDTPA-BOA in A) the brain and B) the liver.

A)

Pre-Contrast

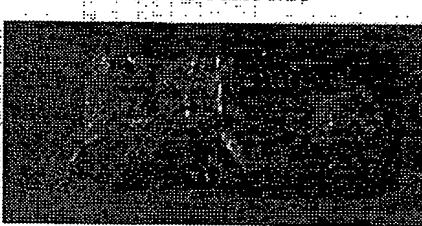


Post-Contrast



B)

Pre-Contrast



Post-Contrast



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Figure 2. Use of GdDTPA-MOA in the Enhancement of Normal Liver

Pre-Contrast



Post-Contrast

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INTERNATIONAL SEARCH REPORT

Intern. Appl. No
PCT/US 95/06818

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K49/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X, Y	MAGN. RESON. MED., 1991, VOL. 19, NO. 2, PAGES 406-15, KABALKA, G. W. ET AL 'Gadolinium-labeled liposomes containing various amphiphilic gadolinium-DTPA derivatives: targeted MRI contrast enhancement agents for the liver' see "Materials and Methods" ---	1-39
X, Y	MAGN. RESON. IMAGING, 1991, VOL. 9, NO. 3, PAGES 373-7, KABALKA, G. W. ET AL 'Gadolinium-labeled liposomes containing amphiphilic Gd-DTPA derivatives of varying chain length: targeted MRI contrast enhancement agents for the liver' see figures see table 1 see page 376, right column - page 377 ---	1-39 -/-

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

11 October 1995

Date of mailing of the international search report

19.10.95

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+ 31-70) 340-2040, Tx. 31 651 epo nl
Fax (+ 31-70) 340-3016

Authorized officer

Dullaart, A

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 95/06818

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,Y	ADV EXP MED BIOL, 1984, VOL. 180, PAGE(S) 835-45, CLARK LC JR ET AL 'Perfluorinated organic liquids and emulsions as biocompatible NMR imaging agents for 19F and dissolved oxygen.' see "CONCLUSION" ---	1-39
Y	WO,A,92 21017 (UNGER EVAN C ;SHEN DEKANG (US)) 26 November 1992 see examples ---	1-39
X,Y	US,A,4 826 673 (DEAN RICHARD T ET AL) 2 May 1989 see examples ---	1-39
X,Y	US,A,4 963 344 (GRIES HEINZ ET AL) 16 October 1990 see examples 2,17,49 ---	1-39
X,Y	US,A,5 120 527 (LI KING CHUEN PETER ET AL) 9 June 1992 cited in the application see the whole document ---	1-39
X,Y	US,A,4 957 939 (GRIES HEINZ ET AL) 18 September 1990 see examples 2,17,49 ---	1-39
P,Y	J MAGN RESON IMAGING, JUL-AUG 1994, VOL. 4, NO. 4, PAGE(S) 631-5, THOMAS SR ET AL 'Evaluation of the influence of the aqueous phase bioconstituent environment on the F-19 T1 of perfluorocarbon blood substitute emulsions.' see abstract see "Materials and Methods" ---	1-39
A	HUM. TOXICOL., 1987, VOL. 6, NO. 6, PAGE(S) 451-458, RAU W. ET AL 'Influence of several chelating agents on the distribution and binding of cadmium in rats' see the whole document -----	1-39

INTERNATIONAL SEARCH REPORT

Int. application No.

PCT/US95/06818

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. Claims Nos.: because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claims 32-39 are directed to a method of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition (rule 39.1(1v)PCT).
2. Claims Nos.: 1-39 because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
please see enclosed sheet .../...
3. Claims Nos.: because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- The additional search fees were accompanied by the applicant's protest.
 No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/210

INCOMPLETE SEARCH...

1. Obscurities,...

Remark: In view of the large number of compounds, which are defined by the general definition and formulae used in claim(s) 1-39, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see guidelines, part. B, chapter III, paragraph 3.6).

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte. nal Application No
PCT/US 95/06818

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9221017	26-11-92	AU-B-	660033	08-06-95
		AU-A-	1998792	30-12-92
		CA-A-	2102605	24-11-92
		EP-A-	0594640	04-05-94
		JP-T-	6507904	08-09-94
		US-A-	5312617	17-05-94
US-A-4826673	02-05-89	NONE		
US-A-4963344	16-10-90	DE-A-	3129906	10-02-83
		DE-A-	3401052	26-07-84
		AU-B-	601916	20-09-90
		AU-B-	1018688	28-04-88
		AU-B-	566007	08-10-87
		AU-B-	8633082	27-01-83
		CA-A-	1218597	03-03-87
		EP-A, B	0071564	09-02-83
		EP-A, B	0169299	29-01-86
		JP-B-	7068193	26-07-95
		JP-A-	62123159	04-06-87
		JP-B-	3039045	12-06-91
		JP-A-	58029718	22-02-83
		JP-A-	3209389	12-09-91
		LU-A-	88291	04-05-94
		US-A-	5021236	04-06-91
		US-A-	4647447	03-03-87
		US-A-	4957939	18-09-90
		US-A-	5362475	08-11-94
		AT-A, B	19184	15-09-93
		AU-B-	607456	07-03-91
		AU-B-	1018488	28-04-88
		AU-B-	574658	14-07-88
		AU-B-	2355984	26-07-84
		BE-A-	898708	16-05-84
		CA-A-	1256249	20-06-89
		CH-A-	660183	31-03-87
		DK-B-	170460	11-09-95
		DK-A-	89794	01-08-94
		FR-A, B	2539996	03-08-84
		FR-A-	2590484	29-05-87

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten
nal Application No
PCT/US 95/06818

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4963344		GB-A- 2137612 GB-A, B 2169598 GB-A, B 2169599 JP-B- 7072162 JP-A- 59139390 JP-A- 4217927 LU-A- 85177 NL-A- 8400079 SE-A- 8400254	10-10-84 16-07-86 16-07-86 02-08-95 10-08-84 07-08-92 24-05-84 16-08-84 22-07-84
US-A-5120527	09-06-92	US-A- 5064636 AU-B- 630748 AU-B- 6472590 CA-A- 2026545 DE-D- 69006164 DE-T- 69006164 EP-A- 0424311 ES-T- 2048477 JP-A- 3188028	12-11-91 05-11-92 26-04-91 20-04-91 03-03-94 05-05-94 24-04-91 16-03-94 16-08-91
US-A-4957939	18-09-90	DE-A- 3129906 DE-A- 3401052 AU-B- 601916 AU-B- 1018688 AU-B- 566007 AU-B- 8633082 CA-A- 1218597 EP-A, B 0071564 EP-A, B 0169299 JP-B- 7068193 JP-A- 62123159 JP-B- 3039045 JP-A- 58029718 JP-A- 3209389 LU-A- 88291 US-A- 5021236 US-A, B 4963344 US-A- 4647447 US-A- 5362475 AT-A, B 19184	10-02-83 26-07-84 20-09-90 28-04-88 08-10-87 27-01-83 03-03-87 09-02-83 29-01-86 26-07-95 04-06-87 12-06-91 22-02-83 12-09-91 04-05-94 04-06-91 16-10-90 03-03-87 08-11-94 15-09-93

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inten. Int'l Application No
PCT/US 95/06818

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US-A-4957939		AU-B- 607456	07-03-91
		AU-B- 1018488	28-04-88
		AU-B- 574658	14-07-88
		AU-B- 2355984	26-07-84
		BE-A- 898708	16-05-84
		CA-A- 1256249	20-06-89
		CH-A- 660183	31-03-87
		DK-B- 170460	11-09-95
		DK-A- 89794	01-08-94
		FR-A,B 2539996	03-08-84
		FR-A- 2590484	29-05-87
		GB-A- 2137612	10-10-84
		GB-A,B 2169598	16-07-86
		GB-A,B 2169599	16-07-86
		JP-B- 7072162	02-08-95
		JP-A- 59139390	10-08-84
		JP-A- 4217927	07-08-92
		LU-A- 85177	24-05-84
		NL-A- 8400079	16-08-84
		SE-A- 8400254	22-07-84